No Association between *TGFBR1*6A* and Lung Cancer

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Introduction: *TGFBR1*6A*, a functionally polymorphic allele in the transforming growth factor β receptor 1 gene (*TGFBR1*), has been hypothesized to increase risk of various cancers. However, little has been documented about connection of this variant with lung cancer.
Methods: In an attempt to explore whether the *TGFBR1*6A* is associated with lung cancer, we performed genotyping followed by sequencing in 252 patients with lung cancer and 250 healthy controls.
Results: The frequency for the heterozygote 9A/6A is 13.9% in cases compared with 12.4% in controls, and the odds ratio is 1.14 (95% confidence interval: 0.68–1.91), which is not statistically significant ($p = 0.62$), suggesting that *TGFBR1*6A* could not be a cancer susceptibility factor for Chinese patients with lung cancer.
Conclusions: We have no evidence to support the hypothesis that *TGFBR1*6A* is associated with lung cancer.

Key Words: *TGFBR1*6A,* Lung cancer, Susceptibility gene, Case-control.

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There is compelling evidence indicating that *TGFBR1*6A* is a functionally polymorphism and plays an important role in initiation and progression of cancer.[1,2] *TGFBR1*6A* may function as a tumor susceptibility allele by switching transforming growth factor β(TGF-β) mediated inhibition of growth into stimulation of growth and also seems to be somatically acquired by primary tumors and metastases.[2] More recently, two meta-analyses for association between *TGFBR1*6A* and cancer risk have shown that *TGFBR1*6A* is emerging as a high-frequency, low-penetrance tumor susceptibility allele in the development of breast, ovarian, and colorectal cancer as well as hematologic malignancies,[3,4] suggesting that this polymorphic allele could be also be associated with an increased risk of other types of cancers including lung cancer. However, no report on whether *TGFBR1*6A* could increase the risk of lung cancer has been found so far. To address this hypothesis, we carried out an unrelated case-control study in the southeast area of China to explore whether the *TGFBR1*6A* is associated with lung cancer.

METHODS

All the cases and controls were from the same geographic region, the southeast area of China, and matched by age and ethnicity (Table 1). Case blood samples were collected after informed consent was provided by 252 patients with a diagnosis of lung cancer in about 2005 (Table 2). This study was approved by the Institutional Review Board of Suzhou University. These 252 specimens include histologically defined adenocarcinoma ($n = 108$), squamous cell carcinoma ($n = 82$), adenosquamous carcinoma ($n = 23$), large cell carcinoma ($n = 19$), and small cell lung cancer ($n = 20$). Most of these patients had not received radiotherapy or chemotherapy before blood sampling. In addition to the case samples, a total of 250 blood samples were also collected from subjects with no history of cancer to be used as controls. Genomic DNA was isolated from the blood samples according to standard proteinase K digestion and phenol-chloroform extraction.

Genotyping was performed on blood samples as described by Samowitz et al.[5] previously. Briefly, 119bp PCR products were amplified from genomic DNA using the primers 5′-ccacaggcggtggcggcgggaccatg-3′ (forward primer) and 5′-cgtcgcccccgggagcagcgccgc-3′ (reverse primer) designed by Pasche et al.[6] Of the two primers, the forward one was labeled with FAM dye at the 5′ end. Following the amplification with guanine cytimidine (GC)-rich buffer (TaKaRa Biotech), polymerase chain reaction (PCR) products were electrophoresed in a 4% acrylamide gel on ABI 3100 Genetic Analyzer (Applied Biosystems), and PCR product size was recognized by means of GeneMapper software. Both *TGFBR1*9A* and *TGFBR1*6A* alleles were further confirmed by TA clone sequencing.

Unconditional logistic regression was carried out to assess the association between *TGFBR1*6A* and lung cancer. Differences were taken be significant at $p < 0.05$. In addition, Hardy-Weinberg equilibrium (HWE) for patients and con-

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TABLE 1. Characteristics of Lung Cancer Patients and Controls

	Cases (n = 252), No. (%)	Controls (n = 250), No. (%)	p^a
Age, yr			0.229
≤55	76 (30.2)	89 (35.6)	
>55	176 (69.8)	161 (64.4)	
Sex			0.486
Male	201 (79.8)	192 (76.8)	
Female	51 (20.2)	58 (23.2)	
Smoking history			0.431
Never	163 (64.7)	171 (68.4)	
Ever	89 (35.3)	79 (31.6)	

[a] For χ^2 test.

TABLE 2. Clinicopathological Features in 252 Lung Cancer Patients in This Study

Clinicopathological Factors	No. (%) of Cases
Tumor size	
T1	38 (15.1)
T2	134 (53.2)
T3	57 (22.6)
T4	23 (9.1)
Nodal involvement	
N0	131 (52.0)
N1	92 (36.5)
N2	19 (7.5)
N3	10 (4.0)
Metastasis	
M0	244 (96.8)
M1	8 (3.2)
Histological types	
Adenocarcinoma	108 (42.9)
Squamous cell carcinoma	82 (32.5)
Adenosquamous carcinoma	23 (9.1)
Large cell carcinoma	19 (7.5)
Small cell lung cancer	20 (8.0)

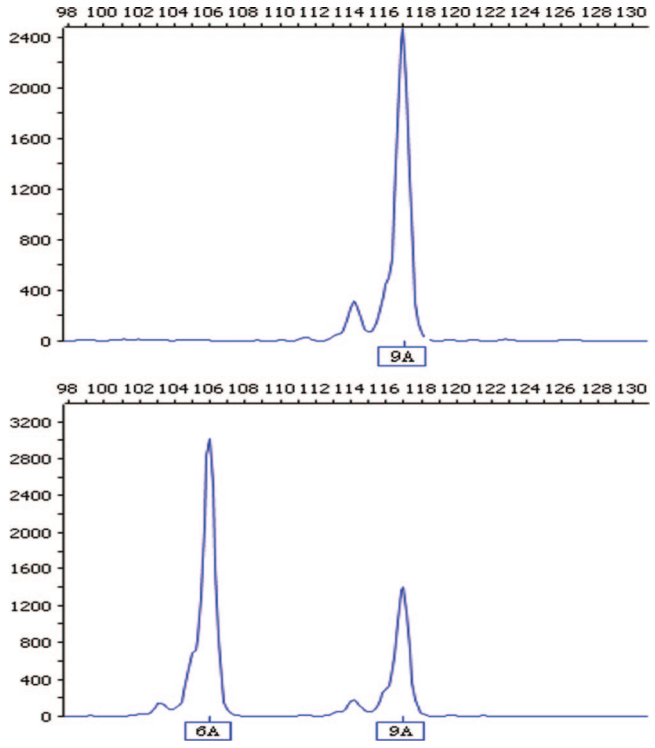

FIGURE 1. Representative electropherograms of the 9A/9A and 6A/9A genotypes. Ineach graph, the scale at the top shows fragment size; the scale on the left shows signalamplitude.

TABLE 3. Genotype Frequencies of the *TGFBR1 9A/6A* among Cases and Controls and Their Contributions to the Risk of Lung Cancer

TGFBR1 Genotype	Cases (n = 252)	Controls (n = 250)	OR (95% CI)	p
9A/9A	217	219	1.00	
9A/6A	35	31	1.14 (0.68–1.91)	0.62
6A/6A	0	0		

OR, odds ratio; CI, confidence interval.

trols was calculated using the DeFinetti program (https://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

RESULTS AND DISCUSSION

As illustrated in Figure 1, two genotypes including 9A/9A and 9A/6A were identified in cases and controls. We found no homozygote for the 6A in both cases and controls, which could be explained by the fact that the *TGFBR186A* allele is not common in populations.[7] The observed frequencies for this polymorphism were in agreement with those expected from the Hardy-Weinberg equilibrium among controls (p = 0.67). Table 3 shows the genotype frequencies, odds ratios (ORs), 95% confidence intervals (CIs), and p values for association between the *TGFBR1*6A* and lung cancer. As shown in Table 3, the frequency for the heterozygote 9A/6A is 13.9% (35/252) in cases compared with 12.4% (31/250) in controls and OR is 1.14 (95% CI: 0.68–1.91), which is not statistically significant (p = 0.62), suggesting that *TGFBR1*6A* could not be a cancer susceptibility factor for Chinese patients with lung cancer. In addition, no association of heterozygote 9A/6A with age, gender, and smoking history was observed in lung cancer patients (Table 4).

Although the frequency of the *TGFBR1*6A* variant has been reported in lung cancer,[1] the case-control analysis regarding this variant remains unclear. Moreover, our previous meta-analysis of *TGFBR1*6A* that included 6968 cases with several types of cancers and 6145 controls showed an overall OR of 1.22 (95% CI: 1.12–1.34) among *TGFBR1*6A* carriers compared with the wild-type *TGFBR1*9A/TGFBR1*9A*.[4] Therefore, we hypothesized an association between *TGFBR1*6A* and lung cancer. In the present study, we found no association of

TABLE 4. Association of *TGFBR1 9A/6A* with Age, Gender, and Smoking History of Lung Cancer Patients

	9A/9A[a]	9A/6A[b]	*p*[c]
Age, yr			0.675
≤55	67	9	
>55	150	26	
Sex			0.473
Male	171	30	
Female	46	5	
Smoking history			0.415
Never	143	20	
Ever	74	15	

[a] Number of cases with 9A/9A.
[b] Number of cases with 9A/6A.
[c] For χ^2 test.

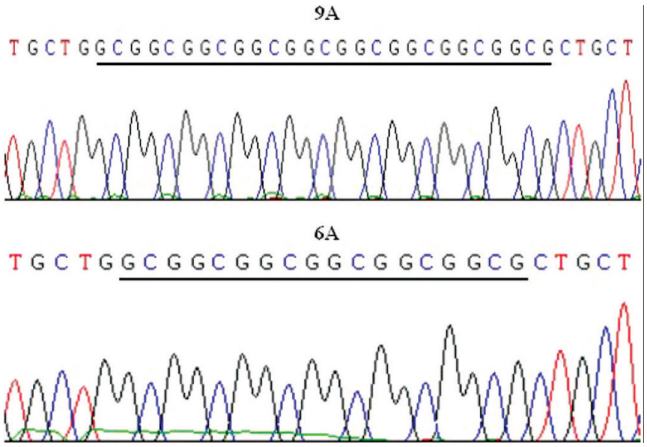

FIGURE 2. Schematic representation of TA clone sequencing of the *TGFBR1*9A* and *TGFBR1*6A* alleles.

*TGFBR1*6A* with lung cancer in China. To exclude the methodological bias and false-negative results for screening *TGFBR1*6A*, we performed TA clone sequencing analysis in parts of samples to confirm the results from genotyping (Figure 2). Data from sequencing were consistent with those from genotyping, indicating that genotyping with the method designed previously by Samowitz et al.[5] was successful and reliable in the present study.

In addition to our null results obtained in lung cancer, Kaklamani et al.[8] did not suggest that the *TGFBR1*6A* predisposes to the development of prostate cancer. What is more, there was no evidence of an association between *TGFBR1*6A* and invasive and postmenopausal breast cancer.[7,9] Taken together, the *TGFBR1*6A* could be a specific susceptibility allele responsible for certain types of cancers, such as colorectal cancer.[10]

In conclusion, we found no evidence to support the hypothesis that *TGFBR1*6A* is associated with lung cancer.

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